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Research Article

FORMULATION AND CHARACTERIZATION OF ALLICIN-AMPHOTERICIN-B LIPOSOMAL GEL FOR THE TREATMENT OF FUNGAL INFECTIONS

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ABSTRACT

Spherical multilamellar vesicles of liposome consisting of lipid egg phosphatidylcholine, soya lecithin and cholesterol were prepared by thin film hydration technique. Further liposomal gel was prepared by egg phosphatidylcholine and cholesterol of molar ratio 7:3 and attained slow drug release with Allicin- Amphotericin B liposomal based gels as compared to the plane gel. Liposomal gel was characterized for drug release kinetics, antifungal activity (disc diffusion assay). Formulation F8 A shows highest drug release. Allicin (*Allium sativum* Linn.) showed a minimum inhibitory concentration (MIC) at the dose range from 0.195 µg/ml to 10 µg/ml of broth against all fungal strains (F2B Formulation). These lower concentrations were achievable for fungistatic effect and the reduced adverse effects. The results suggest that liposomal gel of Amphotericin B may be useful in the treatment of Topical Fungal Infections.

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INTRODUCTION:

Topical liposome formulations are more effective and less toxic than conventional formulations¹. The type and concentration of the polymer, which forms the gel matrix, could influence the stability as well as the release rate of the incorporated drug². Fungal infections are usually more difficult to treat than bacterial infections, because fungal organisms grow slowly and because fungal infections often occur in tissues that are poorly penetrated by antimicrobial agents. Allicin as active constituent of Garlic (*Allium sativum* Linn.), is a bioenhancers capable for improving the bioavailability of drug, reducing the dose of Amphotericin B, adverse reaction and shorten period of treatment³. Combination of Allicin and Amphotericin B show synergistic effect at low concentration. Incorporation of Amphotericin B into Liposome offers a potential means to reduce the toxicity of this antifungal drug⁴.

METHODS:

Preparation of Liposomal gel

Carbopol (934) 2% w/v was allowed to hydrate for 3-4 hr. 5 ml of liposomal suspension was added in gel base and mix by stirrer. The mixture was stirred until thickening and neutralized by the drop wise addition of triethanolamine to achieve the homogeneous gel. The gel was sonicated for 15 minute and then, kept overnight to remove the air bubbles.

Characterization of Liposomal gel

Drug content in liposomal gel

Specific quantity of the prepared gel was taken and dissolved in 100 ml of phosphate buffer of pH 5.5. Volumetric flask containing gel solution was shaken for 2 hr. on mechanical stirrer, filtered and drug absorbance was recorded by UV-visible spectrophotometer at OD 270 nm using phosphate buffer as blank.

In vitro Drug Release

Dialysis membrane was employed in two sides open ended cylinder. One g of liposomal gel containing known amount of drug was placed in a dialysis membrane 2 ml of acetonitrile was added to each aliquot to precipitate the lipids and dissolve the entrapped Amphotericin-B and then the samples were analyzed by UV spectrophotometers at a λ max of 270 nm.

Antifungal Activity

Sabouraud Dextrose Broth (SDB Liquid Medium) Sabouraud Dextrose Agar (SDA Solid Medium) Sabouraud Dextrose Broth (Sabouraud Liquid Medium) was used for cultivation of yeasts, moulds and fungi microorganisms. The sample of strain of *Candida albicans*, *Aspergillus niger*, *Saccharomyces cerevisiae* were cultured and colony forming units were calculated by following formula⁵.

Two fold serial dilution and cup fold method were implied to test for zone of inhibition.

$$CFU/ML = N \times \frac{1}{\text{SAMPLE VOLUME}} \times \frac{1}{D.F.}$$

RESULT AND DISCUSSION:

Surface morphology by Scanning Electron microscopy (SEM)

The samples are examined under scanning electron microscope.



Figure 1: Large unilamellar vesicles

In vitro drug release- Formulation F8 shows high drug release after plane gel.

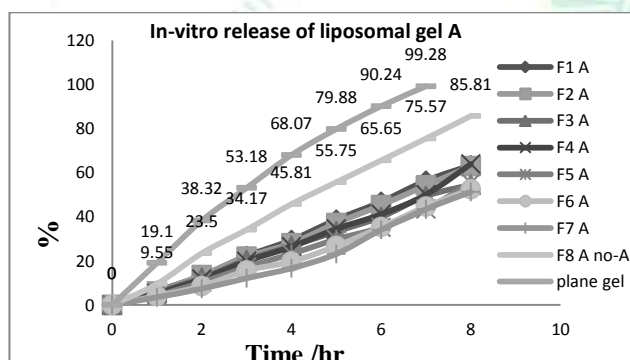


Figure 3: Drug release of liposomal gel (A) \pm S.D. mean (n=3).

Antifungal Activity

The result revealed that the Allicin (*Allium sativum* Linn.) showed a minimum inhibitory concentration (MIC) at the dose range from 0.195 μ g/ml to 10 μ g/ml of broth against all fungal strains. The lowest activity was found with F7A and the greatest activity was found with F2B.

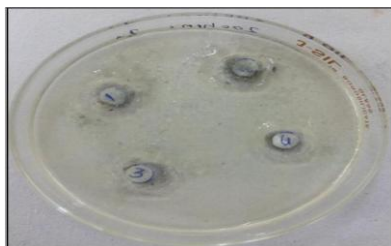


Figure 4: Antifungal activity

Drug content in liposomal gel

The formulated liposomal gel was analyzed for percentage of drug contents Compared to plane gel. The formulation code, F 2 A and F 2 B has showed the highest drug content (84.06 ± 0.33 and $87.05 \pm 0.0.33$).

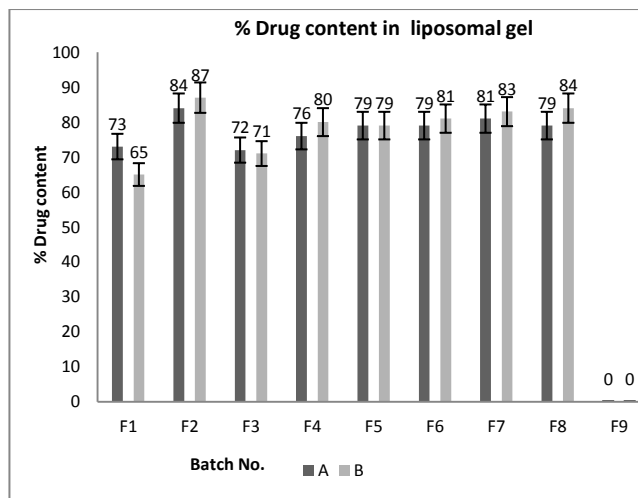


Figure 2: Drug content of liposomal gel \pm S.D. mean (n=3)

CONCLUSION:

Amphotericin B can be successfully incorporated into the liposomal gel formulations. The use of Allicin increase bioavailability and potential to minimize its dose related Adverse Effect of Amphotericin B. Allicin loaded liposomes provide a control release of Amphotericin B. This is due to the robustness and rigid nature of the egg phosphatidylcholine when compared to the soya-lecithin and cholesterol. The liposomal gel shows promising results for targeting of antifungal drugs.

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